The Environmental Stress Response: A Common Yeast Response to Diverse Environmental Stresses

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Abbreviations: ESR, Environmental Stress Response; DTT, dithiothrietol; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; UTR, untranslated region;

ABSTRACT

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Introduction

Unicellular organisms require specific and delicately balanced internal conditions for optimal growth and function. The internal milieu of the cell is maintained to promote proper operation of the cell, however fluctuations in the external environment can result in a variety of cellular perturbations that can disrupt the internal environment. These perturbations can prevent optimal enzyme activities, disrupt metabolic fluxes, destabilize cellular structures, perturb chemical gradients, etc., leading to overall instability. Thus, cells must be able to protect and maintain the critical features of the internal homeostasis in the face of variable external conditions.

Yeast cells have evolved to be very proficient at surviving sudden and often harsh changes in their external environment. In the wild, yeast cells must contend with fluctuations in the temperature, osmolarity, and acidity of their environment, the presence of radiation and toxic chemicals, and long periods of nutrient starvation. Growth under these various conditions requires maintenance of the internal system, however the cellular program required for its maintenance differs depending on the external challenges that the cell must deal with. Thus, when environmental conditions change abruptly, the cell must rapidly adjust its internal milieu to that which is required for growth at the new conditions.

Details regarding the mechanisms that yeast use to adapt to new environments have been emerging over the years. Yeast cells gain cross protection against different stresses, evident by the fact that cells exposed to a mild dose of one stress become resistant to large, normally lethal doses of other stresses (for example [Mitchel, 1982 #199][Blomberg, 1988 #200][Wieser, 1991 #195][Flattery-O'Brien, 1993 #197][Lewis, 1995 #194]. This observation sparked the idea that yeast cells use a general mechanism of cellular protection that is provoked when cells are exposed to stressful stimuli. Concordant with this model was the realization that a set of so-called "heat shock" genes were induced not only by temperature shock but also by other stressful environmental changes, hinting that the genes were involved in generally protecting the cell in response to stressful environments (Kurtz et al. 1986; Werner-Washburne et al. 1989; Kobayashi and McEntee 1990)[Susek, 1990 #198]. Although these observations suggested a general stress response in yeast, the role and regulation of this response remained obscure.

It subsequently became apparent that the expression of the stress-induced genes was controlled by a common mechanism. A number of studies identified a sequence element common to the promoters of the stress-induced genes, referred to as the Stress Response Element (STRE), strongly suggesting that these genes were coregulated by a common factor (Kobayashi and McEntee 1990; Kobayashi and McEntee 1993; Marchler et al. 1993). The hypothetical STRE binding factor was proven to be either of two related zinc-finger transcription factors, Msn2p and Msn4p (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). Deletion of these factors renders cells sensitive to a variety of stressful conditions, and it was subsequently shown that Msn2p and Msn4p govern the induction of a large number of genes in response to many different stresses (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996)[Moskvina, 1998 #204][Boy-Marcotte, 1998 #151]. Thus, these factors became known as the "general stress" transcription factors and were proposed to be generically activated in response to cellular stress to induce a set of genes that defend against environmental insult. It was noted, however, that under certain conditions the targets of these factors were sufficiently induced,

regardless of *MSN2* and *MSN4* deletion [Schuller, 1994 #201], hinting that the regulation of the stress response was more complicated than the initial model suggested.

The recent increase in popularity of whole-genome studies is expanding our definition and understanding of yeast stress responses. Characterization of genomic transcript abundance and global protein synthesis levels are being used to observe the overall cellular responses of yeast cells to environmental changes. Using DNA arrays, the relative transcript levels of all genes in an organism's genome can be rapidly quantified, and computational analysis of the resulting genomic expression programs can implicate gene function and regulation while providing insights into the overall physiological response of the cell (Fodor et al. 1993; Pease et al. 1994; Shalon et al. 1996; Eisen et al. 1998)[Brown, 1999 #202]. Large-scale changes in protein synthesis can be measured by two-dimensional electrophoresis of pulse-labeled proteins, complimenting gene expression studies and adding additional levels of detail about the protein repertoire in the cell (Blomberg 1995; Norbeck and Blomberg 1996; Godon et al. 1998; Lee et al. 1999a)[Appella, 2000 #203]. These types of global studies have provided insights into the mechanisms that yeast use to defend themselves against environmental insult by identifying players in a common response to environmental stress, determining the sensitivity of this response, and suggesting regulatory mechanisms that trigger its initiation. This review will focus on recent advances in defining and studying the common yeast response to stressful environments while summarizing existing literature on the genes and proteins that participate in and regulate this program.

The Environmental Stress Response

Characterization of the genomic expression programs in yeast responding to different environmental conditions reveals that a substantial fraction of each of the responses is not specific to the stimulus but instead represents a common response to all of the conditions tested. In a study by myself and colleagues, we identified approximately 900 genes whose expression was stereotypically altered in response to a variety of stressful environmental transitions (Gasch et al. 2000). (Due to space limitations, the complete list of the genes that participate in this response can be found at http://www-stanford.edu/yeast_stress.) These genes fell into two groups based on their expression patterns (Figure 1): one group consisted of genes whose transcript levels increased in abundance in response to the environmental changes (referred to as induced genes) and the other group was comprised of genes whose transcripts levels decreased following environmental stress (referred to as repressed genes). The two groups of genes displayed nearly-identical but opposite patterns of expression in response to the environmental shifts, strongly suggesting that the expression changes were part of the same cellular program. A similar common gene expression response was also identified in a study by Causton *et al.* (2001), and since then the program has been observed in the cellular response to many environmental conditions, corroborating the commonality of the program (Table 1). Remarkably, the genes that participate in this response amount to ~15% of the currently-predicted genes in the yeast genome (Ball et al. 2000)[Blandin, 2000 #207].

Exploration of the genes involved in this response revealed that many of the induced genes are targets of Msn2p and/or Msn4p (Msn2/4p) and had already been implicated in a general stress response in yeast (Martinez-Pastor et al. 1996; Schmitt and

McEntee 1996). However, characterization of the common gene expression program distinguished it from the previously-described Msn2/4p-dependent response in a number of ways. First, the common gene expression program encompasses nearly 900 genes and includes not only induced genes but also hundreds of genes that are repressed in response to environmental changes. Second, as discussed below in a subsequent section, although many of the induced genes are regulated by Msn2/4p under certain conditions, the coordinate expression changes of these genes extends beyond Msn2/4p control. Furthermore, detailed characterization of the regulation of this response reveals that it is not controlled by a general regulatory mechanism, but rather is mediated by condition-specific signaling pathways. Thus, despite similarities to the previously-defined "general" stress response, the coordinate induction and repression of the genes in this program was referred to as the environmental stress response (ESR) (Gasch et al. 2000). For consistency, the terminology will be maintained in this review.

Responsiveness of ESR gene expression

Each genomic expression program triggered by environmental stress is overall unique to the specific features of the new conditions in terms of the genes affected and the magnitude and choreography of their expression. Nonetheless, the bulk of each genomic expression program is accounted for by the genes in the ESR. The ESR is initiated in response to a wide variety of environmental transitions, as indicated by the stereotyped alterations in expression of the genes in this response (Table 1). Although this program is commonly initiated in response to these diverse conditions, the precise levels and timing of gene expression changes appear to be specific to the environment (Figure 1), hinting at the sensitivity with which the program is regulated.

Like the overall genomic expression responses, initiation of the ESR is often transient: immediately after the shift to a new environment, the cell responds with large changes in the expression of genes in the ESR, however over time the differences in expression usually subside, and transcript levels return to near pre-stress levels (Figure 1) (Gasch et al. 2000; Causton et al. 2001). This observation is in line with previous observations of transient gene expression changes in response to stress (for example [Parrou, 1997 #43; Parrou, 1999 #42][Rep, 1999 #115]. The transient changes in gene expression may help the cell to rapidly adjust the concentrations of the corresponding gene products to the levels required for growth at the new conditions (discussed further below, see Figure 9). According to this model, the transient pattern of gene expression represents an adaptation phase during which the cell optimizes its internal milieu to the new environment.

The magnitude of the expression changes of genes in the ESR is graded to the severity of the environmental shock. Populations of cells experiencing larger doses of stress respond more severely than cells experiencing subtle environmental changes (Gasch et al. 2000). For example, cells exposed to a 25^{o}C to 37^{o}C heat shock show larger and more prolonged changes in gene expression before adapting to their new steady-state expression program, relative to cells exposed to a mild temperature shift of 29^{o}C to 33^{o}C (Figure 2). Furthermore, conditions that result in high levels of cell death provoke a substantial initiation of the ESR, with some of the ESR transcript levels changing greater than 100-fold (A.P. Gasch and P.O. Brown, unpublished data). Thus, the ESR is initiated in response to a wide range of environmental transitions, from subtle changes in

conditions to lethal environmental shocks, in a manner that is graded to the severity of the environmental stress.

The ESR is not initiated in response to any environmental shift but appears to represent a response to suboptimal environments. This is evident from the genomic expression program of cells shifted back and forth between two environments. For example, when cells adapted to growth at 25°C were transferred to 37°C, they responded with large and transient changes in the expression of the ESR genes (Figure 3A) (Gasch et al. 2000). In contrast, when cells adapted to 37°C were shifted to 25°C, they showed reciprocal changes in the expression of these genes: genes that are normally induced during ESR initiation were repressed in response to the reverse temperature shift, and genes that are normally repressed in response to stressful environments became induced under these conditions. This observation indicates that the ESR is suppressed when cells that are adapted to 37°C are shifted to 25°C. Furthermore, the cells immediately (within 5 minutes) adjusted their transcript levels to the final steady-state required for growth at 25°C, with no observable transient features. Thus, while a shift from 25°C to 37°C triggered initiation of the ESR, the reciprocal shift suppressed the stress program with no transient features. A similar result was obtained when cells were shifted back and forth between high and normal osmolarity (Figure 3B): when cells experienced a hyper-osmotic shock, they transiently initiated the ESR before adapting their gene expression program, whereas when cells adapted to high osmolarity were returned to standard medium, they immediately suppressed the ESR to the new steady-state program, with only subtle transience (Gasch et al. 2000).

Collectively, these results reveal that the ESR is initiated when cells are shifted to sub-optimal environments for which their internal features are not optimized. Immediately after a sub-optimal transition, the cell responds with large changes in the expression of its genome, including genes in the ESR, and as the cell adapts to the new environment it adjusts its transcript levels to the new steady-state program. When cells are returned to more optimal environs, the ESR is immediately suppressed with little-to-no adaptation phase, suggesting that the cells can readily adjust when shifted to optimal conditions. This supports the notion that the ESR aids in the protection and adaptation of the cell in response to challenging external environments.

Correlation between changes in transcript levels and changes in protein synthesis

The cell goes to great lengths to alter the expression of its genome, presumably to alter the abundance of the corresponding gene products; indeed, many of the changes in ESR transcript levels correlate with changes in protein synthesis. Proteomic studies have identified proteins whose translation increases or decreases following starvation, osmotic shock, oxidative stress, and heat shock [Fuge, 1994 #5][Norbeck, 1997 #105; Norbeck, 2000 #177][Godon, 1998 #55; Boy-Marcotte, 1999 #205]. Although in each study only a subset of these changes (<100) could be related to specific proteins, ~90% of the reported alterations in translation initiation correlate with the independently-observed changes in transcript abundance [Gasch, 2000 #10](data not shown). Furthermore, it was recently shown that initiation of the ESR parallels the selective translation of transcripts induced in the program. Using DNA microarrays, Kuhn et al. (2001) identified yeast transcripts that displayed altered association with polyribosomes (polysomes) after cells were shifted from glucose- to glycerol-containing medium. Despite an overall reduction in translation

initiation following the environmental shift (indicated by the decreased cellular incorporation of radioactive methionine), essentially all of the 600 transcripts that were increased after the transition, including the ~300 transcripts induced in the ESR, became more associated with polyribosomes, indicating that they were being selectively translated [Kuhn, 2001 #3]. In contrast, ~100 transcripts became less prevalently associated with polysomes after the shift, almost all of which were ribosomal protein transcripts that are strongly decreased as part of the ESR. These results show a strong correlation between gene induction and transcript association with polysomes under the conditions studied, and it is likely that this correlation exists under other conditions as well. Altered polysome association is not a prerequisite for changes in protein synthesis, and thus it is interesting to note that the majority of transcripts that decrease when the ESR is initiated did not show changes in polysome association. The presumed decrease in these gene products may therefore simply be due to the decreased abundance of their transcripts.

Functions represented by genes repressed in the ESR

The precise role that the ESR plays in the adaptation to new environments is suggested by the functional roles of the characterized genes that participate in this response. Approximately 600 genes are repressed in the ESR, and many of them can be directly related to protein synthesis (Table 2). More than 70% of the ~300 characterized genes in this group are annotated by the *Saccharomyces* Genome Database (SGD) as "involved in protein synthesis", and together these ESR genes account for almost 70% of the total number of genes in the yeast genome annotated as such (the exceptions consist almost entirely of genes involved in mitochondrial protein synthesis) (Ashburner et al. 2000; Ball et al. 2000). Given the similarity in the expression patterns of genes repressed in the ESR, many of the uncharacterized genes in this group are most certainly functionally related.

The repression of these genes correlates with the transient decrease in overall translation initiation, coupled with transient cell cycle and growth arrest, that has been observed in the responses to a number of stressful conditions (McAlister and Finkelstein 1980; Fuge et al. 1994; Blomberg 1995; Ashe et al. 2000; Kuhn et al. 2001; Teige et al. 2001). This transient drop in overall translation may be largely caused by the temporary reduction in the levels of these transcripts, coupled with the disassociation of some of the transcripts from polysomes [Ashe, 2000 #4; Kuhn, 2001 #3]; in addition, translation elongation is known to be inhibited during the response to osmotic shock (Teige et al. 2001) and most likely contributes the translation arrest under other conditions as well. The combined effects of the decrease in transcript and protein synthesis may help to conserve mass and energy while the cell adapts to its new conditions. In addition to genes involved in protein synthesis, other functional processes, represented by smaller numbers of repressed genes, include those related to cell wall biosynthesis, cytoskeletal and chaperonin functions, protein glycosylation and secretion, amino acid and pyruvate metabolism, nucleotide biosynthesis, DNA replication, nonsense mediated mRNA decay, and others. Some of the functional categories represented by larger numbers of genes are discussed below.

Ribosome synthesis It is estimated that an actively growing yeast cell harbors ~200,000 ribosomes, each composed of four ribosomal transcripts and 78 ribosomal proteins (RP) (Warner 1999). The rRNA is encoded by 100-200 tandem copies of the rDNA genes and is transcribed primarily by RNA Polymerase (Pol) I to account for approximately 80% of the total RNA in the growing cell. The RP transcripts, generated through RNA Pol II transcription, are estimated to account for more than 60% of the RNA Pol II initiation events, and the synthesized proteins make up 15% of the total mass of cellular proteins under standard growth conditions (Warner 1999).

Because ribosomal synthesis requires substantial mass and energy, it is not surprising that transcript levels of rRNA and RP genes are sharply repressed under stressful conditions. The RP genes are among the most tightly coregulated genes in the yeast genome, and the expression of essentially all of the RP genes is rapidly repressed following environmental stresses, in some cases more than 80 fold (Eisen et al. 1998; Gasch et al. 2000; Causton et al. 2001); in addition, when cells are shifted to a nonfermentable carbon source (and perhaps other conditions as well), the existing RP transcripts become rapidly disassociated from polysomes, further suppressing RP synthesis (Kuhn et al. 2001). Expression of the rDNA is known to be repressed in response to a number of stresses, including heat shock, starvation, secretion defects, and drug treatments (Shulman et al. 1977; Veinot-Drebot et al. 1989; Klein and Struhl 1994; Zaragoza et al. 1998; Nierras and Warner 1999; Miyoshi et al. 2001). Because rRNA levels are infrequently monitored in whole-genome expression studies, rDNA expression in response to diverse conditions has been less thoroughly studied; however, the tight coregulation of the RP genes, and the known importance of the stoichiometry of the ribosomal components (Warner 1989), strongly suggests that the expression of rDNA parallels that of the RP genes and that this repression is part of the ESR.

tRNA synthesis In addition to repression of components of the ribosome, expression of their tRNA substrates is also known to be repressed in response to some stresses. The tRNA transcripts, synthesized by RNA Pol III, are estimated to comprise 15% of the total RNA in a growing yeast cell (Warner 1999). Expression of the tRNAs is known to be repressed following a variety of stresses, including amino acid and nitrogen starvation, progression into stationary phase, defects in secretion, DNA damage, and treatment with rapamycin (Oliver and McLaughlin 1977; Shulman et al. 1977; Kief and Warner 1981; Zaragoza et al. 1998; Li et al. 2000; Ghavidel and Schultz 2001; Pluta et al. 2001). Analogous to the repression of rDNA and RP genes, repression of tRNA synthesis is almost certainly a general feature of the ESR. Consistently, many of the genes involved in tRNA processing, and almost all of the genes encoding cytosolic tRNA synthetases, are repressed as part of this program.

General transcription The decrease in rRNA, RP mRNA, and tRNA levels reveals that genes transcribed by RNA Pol I, II, and III are all repressed in response to stress. This decrease in expression might be expected to correlate with a decreased requirement for RNA Pol I, II, and III activity. Correspondingly, genes encoding RNA Pol I and III are repressed as part of the ESR: all of the genes encoding Pol I and Pol III subunits, as well as most of the shared polymerase subunits, are repressed with patterns similar to the ESR. The decreased expression of these polymerase subunits may contribute to the decreased

expression of their targets, however it should be noted that the rapid decline in rRNA and tRNA levels is likely also regulated at the level of silencing, as is the case of rDNA, and mRNA turnover (Smith and Boeke 1997; Smith et al. 1999). In contrast to the encoded Pol I and III subunits, genes encoding RNA Pol II subunits are generally not repressed in response to stress, nor are Pol II accessory factors such as TFII subunits and the mediator complex. This observation is consistent with the importance of gene induction by RNA Pol II for cell survival of stressful environments.

RNA splicing and export Many transcripts, including rRNA and tRNA transcripts, are synthesized in the nucleolus but must get processed and exported to the cytosol for complete assembly and function (reviewed in Kressler et al. 1999). More than 75% of the genes in the SGD functional category "rRNA processing" (Ashburner et al. 2000; Ball et al. 2000) are repressed in the ESR (including RNA helicases, exoribonucleases, rRNA methyltransferases, and small nuclear ribonucleoproteins), and many genes involved in tRNA processing are also repressed as part of this general stress response. In contrast, only a handful of genes involved in mRNA splicing are repressed in the ESR, despite the fact that nearly half of all of the introns in yeast reside in the RP genes (Warner 1999). Other genes repressed in the ESR are known to be involved in export of the pre-ribosome from the nucleolus, including karyopherins, nuclear transport proteins, and GTP/GDP binding proteins (Stage-Zimmermann et al. 2000), and their decreased expression is likely to be related to the decreased synthesis of ribosomes.

Translation As with transcription, the expression of many genes encoding translation factors is reduced following environmental changes. This repression includes most of the yeast genes encoding translation initiation, elongation, and termination factors, as well as almost all of the cytosolic tRNA synthetases, and correlates with the observed decrease in cellular translation in response to stressful environmental transitions, as discussed above (McAlister and Finkelstein 1980; Fuge et al. 1994; Blomberg 1995; Ashe et al. 2000; Kuhn et al. 2001; Teige et al. 2001). Together, these details indicate that the transient arrest in overall protein synthesis that occurs in response to stressful environmental transitions likely results from a combination of cellular features, including the decreased levels of the transcripts described here, reduced synthesis of translation machinery, direct inhibition of translation elongation, and disassociation of many existing transcripts from polysomes [Ashe, 2000 #4; Kuhn, 2001 #3; Teige, 2001 #8].

Functions represented by genes induced in the ESR

Unlike the genes repressed in the ESR, which can be largely related to protein synthesis and growth-related functions, the genes induced in the ESR are involved in a wide variety of processes. Of the more than 300 genes induced in the ESR, roughly 60% are uncharacterized, indicating that this group is enriched for uncharacterized genes relative to the yeast genome, for which approximately 50% of the genes are of unknown function. The known functions of the characterized genes induced in the ESR have been related to carbohydrate metabolism, metabolite transport, fatty acid metabolism, maintenance of the cellular redox potential, detoxification of reactive oxygen species, autophagy, protein folding and degradation, cell wall modification, DNA-damage repair, secretion, vacuolar and mitochondrial functions, intracellular signaling, and others. The

known functions of these genes hint to the cellular processes that may be affected in response to diverse environmental changes, and suggest mechanisms the cell uses to protect itself in the face of cellular stress. Discussed below are a number of functional categories represented by the characterized genes induced in this program, and the potential cellular effects mediated by the expression changes.

Carbohydrate metabolism A critical component of cell survival is maintaining a viable energy source. Glucose is the preferred carbon source in yeast, and upon stress the cell induces a variety of genes that affect glucose metabolism (Figure 4), including genes encoding glucose transporters that serve to import external glucose into the cell, and glucose kinases that activate the sugar for subsequent catabolism. In response to stressful environments, the fate of glucose is divided between trehalose synthesis, glycogen storage, ATP synthesis through glycolysis, and NADPH regeneration by the pentose phosphate shuttle.

The disaccharide trehalose, comprised of ? -(1,1) linked glucose molecules, has long been implicated in stress responses, particularly the response to hyper-osmolarity, heat shock, and oxidative stress (reviewed in (Singer and Lindquist 1998b; Francois and Parrou 2001). When cells are shifted to high osmolarity, internal trehalose levels (in addition to the well-known osmolyte glycerol) rapidly rise to increase the internal osmolarity of the cell, providing a relatively simple adaptation to high external osmolarity (Hounsa et al. 1998). During heat shock trehalose stabilizes protein structures and prevents aggregation of unfolded proteins (De Virgilio et al. 1994; Hottiger et al. 1994; Singer and Lindquist 1998a), while the sugar appears to protect cellular structures from oxidative damage in response to hydrogen peroxide (Benaroudj et al. 2001). It has also been observed that mutations in the trehalose synthase subunit Tps1p result in defective glycolytic flux, hinting that Tps1p and/or trehalose synthesis may play a role in modulating glycolysis (see more below) (Gonzalez et al. 1992; Van Aelst et al. 1993; Thevelein and Hohmann 1995). The induction of genes affecting trehlose synthesis in response to diverse stresses is well documented, however it has also been observed that this induction does not always correlate with increased trehalose levels. Furthermore, the concomitant induction of genes affecting the synthesis (*TPS1, TPS2, TSL1*) and degradation (*NTH1, ATH1*) of trehalose occurs in response to multiple stresses as part of the ESR (Figure 4) (Parrou et al. 1997; Zahringer et al. 1997; Francois and Parrou 2001). The paradoxical nature of this coinduction was clarified by Zahringer *et al.*(1998), who showed that post-translational regulation of these gene products affects internal trehalose levels: although enzymes synthesizing and degrading trehalose are coinduced, not until phosphorylation and inactivation of the trehlase Nth1p do trehalose levels accumulate in the cell. Regulating these enzymes by phosphorylation provides a method of rapid adjustment of enzymatic activity, and increasing the transcript and protein levels of these enzymes may further expedite the regulation. By coinducing synthetic and catabolic enzymes, the cell can continually modulate the counterproductive enzymatic activities to sensitively control the precise levels of trehalose within the cell (see more below). The importance of precise trehalose levels is underscored by the fact that aberrantly high trehalose concentrations can inhibit protein refolding during the recovery from heat shock (Singer and Lindquist 1998a; Wera et al. 1999).

Storage of glucose in the form of glycogen is known to be critical to cell survival of starvation (ref), and glycogen likely plays an important role in response to a wide variety of stressful environments as well. Similar to the case with trehalose, enzymes leading to the synthesis and degradation of glycogen are coinduced in the ESR (Figure 4) and are post-translationally regulated [Hwang, 1989 #215](Parrou et al. 1997). Both the glycogen synthase Gsy2p and glycogen phosphorylase Gph1p are inactivated by phosphorylation, while Gsy2p is activated by high levels of glucose-6-phosphate. The activity of these enzymes is likely sensitively controlled during stressful situations to modulate glycogen stores. Interestingly, deletion of *GPH1* causes increased glycogen synthesis in response to multiple stresses, suggesting that glucose shuttles into and out of cellular glycogen stores during stress adaptation [Parrou, 1997 #43; Parrou, 1999 #42].

Glucose is catabolized through the glycolytic pathway to synthesize ATP, while generating pyruvate for the TCA cycle; therefore, one might expect that expression changes in genes related to glucose metabolism might include the glycolytic genes. To the contrary, most of the genes encoding glycolytic or gluconeogenic factors do not show expression patterns correlated with the ESR. Instead, genes affecting the synthesis of fructose-2,6-bisphosphate, a key regulator of glycolyic flux, are induced. Both the kinase that synthesizes this allosteric regulator and the phosphatase that degrades it are coinduced as part of the ESR (*PFK26* and *FBP26*, Figure 4). Again, the activity of these enzymes is regulated post-translationally by phosphorylation and by allosteric regulation of Pfk26p and Fbp26p through ATP and fructose-6-phosphate, respectively. The concomitant induction of these synthetic and catabolic enzymes may allow the cell to sensitively modulate glycolysis without altering the expression of the entire pathway of genes.

Fatty acid metabolism Yeast cells can consume metabolites other than glucose to generate energy, including fatty acids. Beta-oxidation of fatty acids occurs in the yeast peroxisome, and the resulting acetyl moieties are then transported to the mitochondria where they supply the TCA cycle for anabolic metabolism and respiration. Although most of the genes involved in fatty acid metabolism are not induced in the ESR, genes involved in importing (*FAA1*, *PXA2*) and exporting (*CAT2*) these metabolites into and out of the peroxisome are induced (Figure 4). The induction of these genes may facilitate fatty acid oxidation simply by increasing the local concentration of fatty acid substrates while efficiently removing the metabolic products for further catabolism in mitochondria. This would allow the cell to take full advantage of existing peroxisomes, rather than expend significant energy required to proliferate the organelles.

Respiration In the presence of glucose, yeast cells are unique among microbes in that they rely on fermentation to generate ATP, despite the fact that respiration generates many more ATP molecules per glucose. Genes involved in respiration are normally repressed in the presence of glucose, however they can be derepressed by a reduced ATP:AMP ratio in the cell, even in the presence of the sugar (Hardie et al. 1998; Carlson 1999; Hardie 1999). As many of the stress defense mechanisms consume ATP, it might be expected that many types of cellular stress would lead to the induction of respiration components. While this is true of the response to environmental changes that lead to substantial cellular stress, genes encoding respiration components are not induced under

all trying circumstances and have an expression profile subtly distinct from the ESR expression pattern (Gasch et al. 2000). Furthermore, although 70% of the genes in this group contain the binding site for the general stress factors Msn2p and Msn4p, fewer than 10% are affected by *MSN2/4* double deletion or overexpression, in contrast to the induced genes classified in the ESR, for which nearly 80% are affected by *MSN2/4* deletion or overexpression (see below) (Gasch et al. 2000). The subtle differences in the expression patterns of the respiration components *versus* ESR genes suggests differences in the regulation of these two responses. Whether the induction of respiration genes is actually part of the general ESR, or is induced concomitantly under some situations by a related regulatory system, remains to be characterized in detail.

The cell does, however, induce as part of the ESR a handful of genes that have been implicated in respiration. For example, in response to diverse stresses the cell induces genes encoding the rate-limiting step of the TCA cycle (*CIT1*), an alternate isoform of cytochrome C (*CYC7*), and two factors that affect the assembly and synthesis of the oxidative phosphorylation components cytochrome C oxidase (*COX15*) and ubiquinone (*COQ5*). Increasing the levels of these gene products may promote ATP synthesis by utilizing existing respiration components. Alternatively, the induction of cytochrome C and ubiquinone may play a role in the defense against oxidative stress, as discussed below, rather than in ATP generation.

Oxidative stress defense Reactive oxygen species (ROS), generated endogenously through oxidative phosphorylation and enzymatic activities or exogenously by environmental factors, can lead to a chain of oxidation reactions in the cell, damaging cellular structures such as proteins, lipids, and DNA, and preventing proper enzymatic activity by perturbing the internal redox potential (see Scandalios 1997) and Chapter X for review). Thus, the cell has evolved a number of defenses against oxidative stress as well as mechanisms to maintain its internal redox potential.

The reducing environment of the yeast cytoplasm is affected by the balance of oxidized and reduced protein sulfhydryl groups, including those of the small peptide glutathione (Grant et al. 1996b; Grant 2001). This balance is modulated by thioredoxin and glutaredoxin, which mediate the NADPH-dependent reduction of disulfide groups. During oxidative stress, the oxidation of glutathione and other thiol-specific antioxidants, coupled with subsequent reduction by thioredoxin and glutaredoxin, is thought to help buffer the reducing environment of the yeast cytoplasm while protecting cellular structures from oxidative damage (Kuge and Jones 1994; Grant et al. 1996a; Grant et al. 1996b; Luikenhuis et al. 1998). The importance of these cellular features likely explains the induction of many of these genes in response to diverse environmental stresses: as part of the ESR, the cell induces genes encoding isozymes of thioredoxin and glutaredoxin (*TRX2*, *TTR1*), the glutathione-biosynthetic enzyme gamma-glutamyl transferase (*ECM38*), and thiol-specific antioxidants (*PRX1*, *YDR453C*). Furthermore, genes encoding the NADPH-generating steps of the pentose phosphate shuttle (*ZWF1*, *GND2*) are also induced as part of the ESR, perhaps to help replenish NADPH reducing equivalents (Juhnke et al. 1996; Slekar et al. 1996).

Yeast cells also utilize a number of enzymes devoted to the detoxification of ROS, and many of these are induced not only by conditions that inflict oxidative damage but also in response to diverse stressful environments. Among those that get induced are

genes encoding the cytosolic superoxide dismutase Sod1p and cytosolic catalase Ctt1p, which reduce superoxide and hydrogen peroxide, respectively. While catalase appears to specifically reduce hydrogen peroxide, the glutathione peroxidases Hyr1p and Gpx1p may act on organic peroxides as substrates, and the genes encoding these enzymes are also induced in the ESR (Inoue et al. 1999; Avery and Avery 2001).

Under standard growth conditions, endogenous ROS is thought to be generated primarily by electron leakage from the oxidative phosphorylation chain in mitochrondia [Scandalios, 1997 #64], and therefore it is not surprising that this organelle harbors its own local ROS protection systems. Cyctochrome b5 reductase (*MCR1*) and cytochrome C peroxidase (*CCP1*) both seem to protect against oxidative stress, as deletion of either of their genes increases the sensitivity of yeast cells to drugs that induce oxidative damage (Charizanis et al. 1999; Lee et al. 2001a); both genes are induced with the ESR. Oxidized cytochrome C peroxidase is subsequently reduced directly by cytochrome C (ref). With this in mind, the induction of the cytochrome C isoform Cyc7p in the ESR may be related to Ccp1p reduction instead of respiration. Another respiration component, ubiquinone, may also play a role in oxidative stress: this membrane-diffusible molecule can act as an antioxidant against lipid peroxides, and indeed, yeast cells defective in ubiquinone synthesis are sensitive to exogenously added peroxides (Schultz and Clarke 1999; Soballe and Poole 1999). One gene required for Ubiquinone synthesis is induced in the ESR (*COQ5*), although other genes involved in its synthesis do not appear to be generally induced.

Autophagy and vacuolar functions Autophagy is a process whereby the cell randomly engulfs its cytoplasmic material into vesicles destined for the yeast vacuole (reviewed in Abeliovich and Klionsky 2001). The engulfed materials, including proteins, small molecules, and even organelles, are degraded in the vacuole so that their components can be recycled in the cell. Although autophagy shares many features with the constitutive process of cytoplasm-to-vacuole trafficking, which trafficks specific cytoplasmic proteins, the process of autophagy is specifically induced by starvation [Takeshige, 1992 #208; Tsukada, 1993 #80][Abeliovich, 2001 #78]. Interestingly, genes involved in autophagy (*APG1, APG7, AUT1*), including the Apg1p kinase that may regulate the process (Tsukada and Ohsumi 1993; Matsuura et al. 1997)[Kamada, 2000 #196], are induced as part of the ESR in response to a wide variety of stresses, while genes specific to cytoplasm-to-vacuole trafficking are not commonly induced (Gasch et al. 2000). Although it is not known if the induction of these genes necessarily leads to increased autophagy, recent evidence from Wang *et al.* (2001) reveals that autophagy is active at the diaxic shift, with timing that parallels the induction of the autophagy genes in the ESR (DeRisi et al. 1997; Wang et al. 2001a). Whether autophagy trafficking is activated in response to stresses beyond starvation is currently unknown.

In addition to autophagy genes, genes encoding vacuolar proteins are also induced in the ESR. These include genes encoding the vacuolar calcium pump Pmc1p, a protein implicated in vacuolar targeting (*VAB2*), a variety of vacuolar proteases (*PRC1, YPS6, LAP4, PEP4, PRB1*) as well as the protease inhibitors Pai3p and Pbi2p which are thought to inhibit the Pep4p and Prb1p proteases, respectively. The products of these genes may aid in the degradation of cargo delivered to the vacuole by autophagy as well as other

proteins that arrive through endocytosis or through the secretion pathway (Van Den Hazel et al. 1996).

Protein folding and degradation Many protein folding chaperones are known to be induced specifically in response to heat-denatured proteins (see Chapter X for review), however it was early realized that a subset of chaperone genes are induced by a variety of stressful conditions (Kurtz et al. 1986; Werner-Washburne et al. 1989). The chaperones induced as part of the ESR include the so-called small heat shock proteins (*HSP12*, *HSP26*, and *HSP48*), members of the Hsp70 family of chaperones (*SSA4*, *SSE2*, *HSP78*), and *HSP104*.

The functions of the small heat shock proteins are not well characterized, however Hsp26p was shown to protect proteins from heat-denaturation *in vitro* (Haslbeck et al. 1999), while Hsp42p has been implicated in repolarization of the actin cytoskeleton after adaptation to elevated temperatures (Gu et al. 1997). Individually, these chaperones appear to contribute little to thermotolerance in yeast, as deletion of the any of the factors does not result in cellular sensitivity to heat shock or other conditions (Susek and Lindquist 1989; Gu et al. 1997). On the other hand, deletion of the *HSP104* chaperone gene does affect viability during heat shock (Lindquist and Kim 1996). Hsp104p appears to help disassociate aggregates of unfolded proteins to allow the Hsp70 chaperones, perhaps including Ssa4p and Sse2p that are induced in the ESR, to bind and refold the protein substrates (Parsell et al. 1994; Glover and Lindquist 1998). Similar to the Hsp104-Hsp70 system in the cytoplasm, the mitochrondrial Hsp70 homolog Hsp78p has properties similar to both Hsp104p, to which it is 46% identical, as well as Hsp70 chaperones (Schmitt et al. 1995; Schmitt et al. 1996). Thus, chaperones that are localized to the cytosplasm and mitochrondria are induced in the ESR, underscoring the importance to stress survival of proper protein folding in these subcellular locations.

Denatured proteins that can not be properly refolded are targeted for degradation by ubiquitination, and thus it is not surprising that genes involved in ubiquiting targeting are also induced in the ESR. Genes encoding E1 and E2 proteins involved in ubiquitin ligation and conjugation are induced in the ESR (*HUL4*, *UBC5*, *UBC8*), as is *UBI4*, which encodes polyubiquitin, and *UBP15*, which encodes a putative deubiquitinating enzymes. Deletion of *UBC5*, *UBI4*, or *UBP15* is known to render cells sensitive to a variety of stresses (Finley et al. 1987; Seufert and Jentsch 1990; Spence et al. 1995; Amerik et al. 2000), highlighting the importance of ubiquitin-dependent processes in the cell. Ubiquitination is important not only for removing misfolded proteins from the cell but also for targeting active proteins for turnover, and the induction of ubiquitin-mediated protein degradation may help the cell to rapidly alter the internal protein repertoire during stress adaptation. Monoubiquitination is specifically involved in targeting proteins for endocytosis and altering histone structure to affect chromatin remodeling (Hicke 1999; Hicke 2001), and thus the induction of some of the genes involved in ubiquitin metabolism may also play a role in these processes.

Cytoskeletal reorganization When actively growing cells progress through the cell cycle, the arrangement of their actin cytoskeleton is polarized so that vesicles and materials can be delivered along the actin cables to the site of cell growth at the bud neck (reviewed in Pruyne and Bretscher 2000). In response to cellular stress, however, the actin

cytoskeleton becomes rapidly depolarized and instead arranges isotropically, perhaps to deliver secretory vessicles and their cargo evenly within the cell (ref). Soon after the adaptation to the new environment, the cytoskeleton becomes repolarized to promote localized growth and subsequent cell division. Reorganization of the cytoskeleton may be affected by factors encoded by genes in the ESR: a number of cytoskeletal genes are repressed as part of the ESR, including those encoding Pac10p, a protein involved in microtubule assembly whose deletion renders cells sensitive to the actin destabilizing drug latrunculin (Geissler et al. 1998), and Sro9p which is proposed to affect the organization of actin fibers (Kagami et al. 1997); two genes induced in the stress program have also been implicated in cytoskeletal function, including the genes encoding Arc18p, a member of the Arp2/3 complex that is thought to affect the mobility and polar distribution of cortical actin patches (Winter et al. 1997; Machesky and Gould 1999; Winter et al. 1999), and Hsp42p, which plays a role cytoskeletal repolarization after heat shock (Gu et al. 1997). In addition, Msn2/4p have been specifically linked to cytoskeletal reorganization following cellular stress: repolarization of the cytoskeleton is partially controlled by Ras-dependent signaling, and the defective cytoskeletal reorganization seen in *ras*? mutant cells can be suppressed by deletion of *MSN2/4* (Ho and Bretscher 2001). One explanation for this result is that genes induced by Msn2/4p inhibit cytoskeletal rearrangements, and therefore deletion of *MSN2/4*, and reduced expression of their target genes, suppresses the *ras*? cytoskeletal phenotype. However, an alternative function of Msn2/4p may be one of physical interaction: Msn2p and Msn4p were recently shown to bind Bmh2p (Beck and Hall 1999), a protein that has been linked to RAS/PKA-dependent signaling (Gelperin et al. 1995; Roberts et al. 1997; Roth et al. 1999) and affects the organization of the actin cytoskeleton (Roth et al. 1999). Although there is no direct evidence of a physical association between Msn2/4p and the actin cytoskeleton, the possibility exists that Msn2/4p play a more direct role in affecting cytoskeletal rearrangements.

Signaling A variety of genes involved in cellular signaling are induced in the ESR, and many of these genes can be related to specific signaling pathways. Most notably, genes affecting Protein Kinase A (PKA) signaling are induced as part of the ESR. Both positive and negative regulators of PKA signaling are coinduced in the ESR (Figure 5). Two of the three catalytic subunits of PKA (*TPK1, TPK2*) are induced concomitant with the cAMP-dependent inhibitory subunit, *BCY1*, the phosphodiesterase *PDE1*, and the gene encoding the Yak1p kinase that is proposed to counteract PKA signaling (Garrett and Broach 1989; Hartley et al. 1994). The induction of PKA signaling components is intriguing, since activity of this pathway is known to suppress the stress response by triggering the cytoplasmic relocalization of Msn2/4p and by activating expression of the RP genes (Gorner et al. 1998). Even more interesting is the finding that stress-dependent induction of the PKA subunits is dependent on Msn2p and Msn4p (Gasch et al. 2000). Analogous to PKA, the Tor1p kinase negatively regulates Msn2p and Msn4p activity by affecting their localization (Beck and Hall 1999), and the *TOR1* gene is also induced in the ESR in a manner dependent on Msn2/4p. As discussed below in a subsequent section, the induction of these genes as part of the ESR may aid in the efficient suppression of the ESR after the cell has adapted to new conditions.

Functional themes in the ESR

Differential expression of isozymes One notable feature of the ESR is the differential expression of isozymes: many genes involved in carbohydrate metabolism, respiration, protein folding, oxidative stress, and other processes are induced as part of the ESR, while their paralogous counterparts are not (Table 3) (Norbeck and Blomberg 1997)(Gasch et al. 2000; Rep et al. 2000; Causton et al. 2001). This divergence in expression may result from the different properties of the putative enzyme pairs, including biochemical function, substrate specificity, and physical location. For example, it has been shown that yeast glutathione peroxidases and thiol-specific antioxidants have different substrate specificities (Inoue et al. 1999; Lee et al. 1999b; Avery and Avery 2001), and thus the activity of some of these gene products may be critical for cell survival in response to certain conditions, while others are commonly involved in stress defense. In the case of yeast catalases, differences in their localization may explain the general induction of the cytosolic catalase Ctt1p as part of the ESR, while the peroxisomal catalase Cta1p is specifically induced under conditions of high peroxisomal activity (Filipits et al. 1993).

An alternative model for the differential expression of isozymes possessing similar properties is that it provides a mechanism of differential regulation of enzyme activity. Duplication of these genes and divergence of their regulatory mechanisms may have provided yeast a way to differentially regulate the encoded enzymatic activity. This might allow the cell to regulate ESR isozymes as part of this general program, while their counterparts are regulated by specialized signals.

Coinduction of genes with counterproductive functions A recurring theme in the ESR is the induction of genes that encode counterproductive activities. This can be seen by the concomitant induction of genes leading to the synthesis and degradation of trehalose, glycogen, and fructose-2,6-bisphosphate, the coinduction of vacuolar proteases and their inhibitors, and the coregulated increase in transcripts encoding positive and negative effectors of cellular signaling. Many of these gene products are known to be regulated by post-translational regulation. Thus, the coinduction of gene products may render the cell poised to rapidly modulate enzymatic activity post-translationally. This would allow the cell a mechanism of rapid enzymatic regulation while alleviating the delay required for nascent protein synthesis.

Subtle modulation of the activities of positive and negative regulators may buffer concentrations of critical metabolites in the cell. A good example of this is the case of glucose. During stress conditions, the cell must regulate the flux of glucose into trehalose generation, glycogen synthesis, the pentose phosphate shunt, and glycolysis (Figure 4). Inappropriate catabolism of glucose might deplete the sugar from critical metabolic pathways, while too much free phosphorylated glucose may lead to detrimental allosteric effects of enzymes regulated by this metabolite. Thus, the cell may buffer its intracellular glucose levels by the continued flux into and out of its trehalose and glycogen stores. This hypothesis is supported by the observation that cells lacking the catalytic subunit of trehalose synthase (Tps1p) have aberrantly high levels of fructose-1,6-bisphosphate, due to unregulated flux of glucose into glycolysis, and thus cannot grow on glucose-containing medium (Gonzalez et al. 1992; Van Aelst et al. 1993; Thevelein and Hohmann

1995) Although the mechanism of this Tps1p-dependent effect is unknown, it is intriguing to consider that it results from unbuffered glucose pools (Thevelein and Hohmann 1995; Francois and Parrou 2001).

Regulation of control steps of metabolic processes Another recurring feature of the ESR is the regulation of genes whose products affect regulatory or rate-limiting steps of metabolic processes, rather than the altered expression of entire pathways of genes. For example, as part of the ESR the cell induces *CIT1* and *ZWF1*, encoding enzymes that perform the rate-limiting steps of the TCA cycle and pentose-phosphate shuttle, respectively, without significantly affecting other genes in these pathways. Similarly, the induction and subsequent regulation of two enzymes modulating fructose-2,6-bisphosphate may be enough to sensitively control glycolysis *versus* gluconeogenesis without altering the expression of most of the genes directly involved in these pathways. Increased expression of transporters, including plasma membrane glucose transporters, peroxisomal fatty acid transporters, and a mitochondrial acyl carrier protein, may affect metabolic flux simply by altering the local concentrations of the substrates and products of the pathways. These examples suggest that through initiation of the ESR the cell may attempt to affect specific cellular processes through few and simple expression changes. This would not only reduced the effort and energy required to modulate those processes in response to stressful environments, but would also facilitate the rapid return to normal growth after the stress has been alleviated.

The Role of the ESR

The ESR represents a common gene expression program that is initiated in response to a wide variety of sub-optimal growth conditions, in a manner that is graded to the severity of the environmental stress. Based on these observations, we proposed that ESR is initiated to protect critical features of the internal homeostasis while the cell adapts to new environments (Gasch et al. 2000). When the cell is shifted to an environment for which its internal system is not optimized, the specific cellular consequences resulting from the shift can lead to a series of secondary instabilities within the cell, potentially threatening many key physiological systems. To prevent such wide-spread internal damage, the cell has evolved to initiate the ESR in response to diverse signals of cellular stress in order to protect and maintain these critical features of the internal system.

The protective nature of this program is consistent with the observation that a number of the gene products induced in the ESR are not necessarily active, supporting the notion that they are induced in preparation for their potential activity. Many of the gene products discussed here are regulated at the post-translational level, and thus induction of the genes renders the cell poised to rapidly regulate their products. Another indication that some of the ESR enzymes are not always highly active is suggested by the fact that genes encoding their cofactors are not part of the ESR but rather are induced under specific conditions. For example, although genes encoding thioredoxin and glutaredoxin are induced in the ESR, the genes encoding their cofactors, thioredoxin reductase and glutathione reductase, are only induced in response to conditions of severe oxidative stress, when the activity of thioredoxin and glutaredoxin is required for cell survival (Gasch et al. 2000)(Kuge and Jones 1994; Grant et al. 1996a; Luikenhuis et al.

1998). Finally, it has been observed for many of the induced ESR genes that their deletion does not result in a general sensitivity to all stresses but renders the cell sensitive to specific conditions only, revealing that those gene products are only required under some situations **(**for example Ramotar and Masson 1996; Parrou et al. 1997; Broomfield et al. 1998; Bruning et al. 1998; Luikenhuis et al. 1998; Thomson et al. 1998). Thus, initiation of the ESR serves to prepare the cell to rapidly utilize the synthesized gene products in the event that they are required to protect features of the physiological system.

Considering the known functions of characterized genes in the ESR points to internal features that are protected in sub-optimal environments. Most of the genes repressed in the ESR can be related to protein synthesis, and the reduction in these transcripts, coupled with transient growth and cell cycle arrest, may help to conserve mass and energy while the cell adapts to the challenges of its new environs (Warner 1999; Gasch et al. 2000). The genes induced in the ESR are involved in a wide variety of processes, as discussed above, and the induction of these genes may prepare the cell to protect critical features of the internal homeostasis, such as internal glucose stores, ATP levels, internal osmolarity, the cellular redox potential, the integrity of cellular structures such as proteins and DNA, and other features. Indeed, the initiation of the ESR probably explains the observed cross protection again stressful environments, in which cells exposed to a low dose of one stress become resistant to an otherwise-lethal dose of a second, unrelated stress [Mitchel, 1982 #199][Blomberg, 1988 #200][Wieser, 1991 #195][Flattery-O'Brien, 1993 #197][Lewis, 1995 #194].

Regulation of ESR gene expression

Although to a first approximation the ESR is commonly initiated in response to many stressful environments, several lines of evidence suggest that genes in this program are not controlled by a single system but are governed by different mechanisms in response to different environmental conditions. Subgroups of genes in the ESR display subtly different expression patterns, relative to other ESR genes, in response to specific stimuli. For example, genes involved in oxidative stress defense are induced as part of the ESR but are super-induced in response to conditions that result in oxidation (Figure 6). Similarly, although genes encoding protein folding chaperones are induced in the ESR, they are more highly induced in response to conditions of protein unfolding, relative to other genes in this program. These subtleties also apply to genes that get repressed in the ESR. In many of the experiments observed, transcripts of the RP genes decrease concomitantly with the other repressed ESR genes, however in response to certain experiments, such as exposure to the sulfhydryl oxidant diamide, the RP genes are decreased with a delayed and muted profile, relative to the non-RP genes in the ESR. Thus, implications of condition-specific regulators are evident in the expression patterns of both the induced and repressed ESR genes.

A variety of regulatory mechanisms have been implicated in the regulation of genes in the ESR. Although some of the regulatory factors that contribute to the regulation of the ESR are known (Table 4), the complexity of this system is only beginning to unfold. Discussed below are some of the key regulatory factors and systems that have been implicated in mediating ESR gene expression, followed by a model for the condition-specific regulation of the ESR.

Rap1p The multipurpose factor Rap1p has been implicated in a variety of cellular processes, and as a transcriptional activator it is responsible for the high level of expression of the RP genes in actively growing cells (reviewed in Morse 2000). Because the turnover of these transcripts is relatively fast (5-10 minutes at standard conditions), constant Rap1p-mediated transcription helps to maintain these transcripts at high levels in the cell (Li et al. 1999). However, in response to stressful conditions, RP transcripts rapidly decline, in concert with other genes repressed in the ESR. The mechanism of RP gene repression in response to heat shock appears to be mediated through silencing, coupled with the constitutively high rate of turnover of the RP transcripts (Li et al. 1999). However, the mechanism appears to be condition-specific and in some cases dependent on Rap1p: in addition to its role as a transcriptional activator, Rap1p also mediates gene silencing by interacting with a host of silencing factors through its carboxyl-terminal silencing domain (Morse 2000). Li *et al.* showed that the decrease in RP transcripts following DTT treatment required the silencing domain of Rap1p, while the drop in RP transcript levels after heat shock was independent of this Rap1p domain (Li et al. 1999); a similar result was obtained by Miyoshi *et al.* (2001), who demonstrated that silencing of the rDNA was dependent on the Rap1p silencing domain in response to DTT but not during nitrogen starvation (Miyoshi et al. 2001). This result reveals that, at least in some cases, Rap1p plays multiple roles in regulating expression of the RP genes, and that different mechanisms govern the repression of these genes under different circumstances.

Many of the promoters of RP genes contain multiple binding sites for Rap1p, and these promoters were recently shown to be bound by Rap1p *in vivo.* Lieb *et al.* (2001) performed chromatin immuno-precipitation experiments to identify the genomic sites bound by the factor in living cells (Lieb et al. 2001). In addition to the RP gene promoters, the promoters of roughly 15 other genes that are repressed in the ESR, many of them involved in rRNA processing, were also bound by Rap1p. Most of the Rap1p-bound promoters contain binding sites for the factor; in addition, ~80 non-RP genes repressed in the ESR also contain Rap1p binding sites in their promoters but were not observed to be bound by Rap1p under standard growth conditions. These data implicate Rap1p in regulating other genes that are repressed in the ESR; however, the expression pattern of most of these genes is subtly distinct from that of the RP genes, suggesting that their repression is mediated by a distinct mechanism.

In addition to promoters of repressed ESR genes, Rap1p was also detected bound to the promoter regions of a handful of genes normally induced in the ESR (Lieb et al. 2001). The promoters of 13 induced ESR genes were bound by Rap1p, and included in this set was *GPD1*, whose induction in response to osmotic shock is Rap1p-dependent (Eriksson et al. 2000). Promoter analysis of this set of 13 genes revealed that 70% of them contain a novel sequence, AAAGGAG, that may be involved in the regulation of these genes. Although the putative binding factor of this sequence is not known, a candidate regulator is the recently identified transcription factor Hot1p. This factor appears to be induced specifically in response to hyper-osmotic shock, and it is known to regulate a number of genes induced in the ESR under these conditions, including *GPD1* (Rep et al. 1999; Rep et al. 2000). Because Hot1p shares sequence homology with the transcription factor Gcr1p, which acts with Rap1p to regulate genes involved in glycolysis, it was suggested that Hot1p may also function with Rap1p to regulate its

targets (Rep et al. 1999). That the sequence motif identified in the Rap1p-bound promoters differs from the known Gcr1p site at one position (gAAGGAG) (ref) raises the possibility that it may represent the unknown binding site of Hot1p. Regardless, the data of Lieb *et al.* clearly point to a role for Rap1p in the regulation of some of the genes induced in the ESR.

Chromatin remodeling In addition to modulating transcription initiation, the cell can mediate the expression of its genome by altering chromatin structure to silence or desilence genes. In yeast, chromatin remodeling is affected by ATP-dependent chromatin remodeling factors as well as enzymes that chemically modify histones (reviewed in (Perez-Martin 1999; Peterson and Logie 2000). The factors that mediate these processes are recruited to the sites of action by specific DNA binding proteins that bind to conserved sequences in the genome. The yeast corepressors Tup1p/Ssn6p have been proposed to act as bridging factors between these specific DNA binding proteins and histone modifying factors, including the histone deacetylaces Rpd3 and Hda1p (Smith and Johnson 2000; Watson et al. 2000; Wu et al. 2001). The resulting alterations in chromatin structure are though to affect gene expression in the vicinity of the structural changes.

A number of lines of evidence suggest that the general repressors Tup1p/Ssn6p may contribute to the regulation of some of the genes that participate in the ESR. Under conditions of normal cell growth, the site-specific repressor Sko1p maintains the expression of genes required for growth at high osmolarity, including a number of genes that are normally induced in the ESR (*GRE2, YML131W, GPD1, ALD2, DDR48*); these genes become derepressed after a hyper-osmotic shock in a manner dependent on the osmolarity-induced Hog1 pathway (Proft et al. 2001; Rep et al. 2001). Numerous indications suggest that Tup1/Ssn6p are involved in this repression: *ssn6* and *tup1* mutant cells show higher transcript levels of the Sko1p targets, and deletion *SSN6* suppresses the NaCl sensitivity of *hog1* mutants (Garcia-Gimeno and Struhl 2000; Rep et al. 2001). Deletion of *TUP1* also leads to the derepression of many genes, including those that participate in the ESR, as measured in microarray experiments (DeRisi et al. 1997). Roughly 60 genes that are normally induced (such as *GRE2, YML131W, DDR48*, and others) or repressed (including the RP genes) under stressful situations were derepressed in the *tup1* mutant cells, including the gene encoding Msn4p. (It should be noted that a significant fraction of genes that are normally induced or repressed in the ESR were expressed at lower levels in the *tup1* cells, for reasons that are not understood.) One caveat of these experiments is that deletion of the general repressor Tup1p may be stressful to cells and may therefore indirectly trigger ESR initiation. An argument against this situation is that the genomic expression program in *tup1* mutant cells does not resemble the stereotyped ESR expression pattern: only a subset of the ESR genes was affected by *TUP1* deletion, and the genes that were affected did not display the stereotyped expression profile seen during ESR initiation. Therefore, these data suggest that Tup1p and Ssn6p play multiple, direct roles in affecting the expression of both induced and repressed genes in this program. These factors may contribute to the repression of some of the ESR genes when the program is initiated, but they may also aid in ESR suppression under standard growth conditions by mediating the silencing of genes that are induced in the program under conditions of stress.

Other data suggest that genes that are induced or repressed in the ESR in response to stressful environments are regulated by chromatin remodeling factors. The most complete data comes from a recent study by Fazzio *et al.* (2001) that characterized genomic expression in cells defective in the activities of a number of chromatin remodeling proteins (Fazzio et al. 2001). They observed expression in cells deleted for the histone deacetylase Rpd3p and the chromatin remodeling factor Isw2p, two proteins thought to act in parallel pathways to mediate the repression of meiosis-specific genes (Goldmark et al. 2000). In addition, the group also observed genomic expression in cells harboring catalytically inactive mutants of Rpd3p and Isw2p, as inactive Rpd3p alleles provoke dominant-negative affects in cells (Kadosh and Struhl 1998). Double deletion of both factors resulted in the derepression of many genes, including ~30% of the genes normally induced in the ESR; the remaining ESR genes were largely unaffected in terms of expression. Most interestingly, cells harboring the catalytically inactive Rpd3p, but not the Isw2p inactive mutant, showed derepression of a different set of genes – most of these genes are normally repressed in the ESR, and together they amount to roughly 30% of the repressed ESR genes. Again, the data suggest that these effects were specific to these subsets of ESR genes, as opposed to a general initiation of the entire ESR as a secondary response to Rpd3p and Isw2p defects. The genes that were derepressed in cells harboring the inactive Rpd3p mutant largely overlap a set of non-RP ESR genes that contain the promoter sequence GATGAG, although it is unknown if this promoter element has anything to do with the Rpd3-dependent regulation of these genes (Hughes et al. 2000a; Gasch et al. 2000; Fazzio et al. 2001). Most of the genes that were derepressed by deletion of *RPD3* and *ISW2* did not correlate with the genes affected by *TUP1* deletion, indicating that, although Tup1p recruits Rpd3p under certain situations, the proteins can act independently of each other. Interestingly, many of the genes that were derepressed by the catalytically inactive Rpd3p mutant were slightly repressed by deletion of *TUP1*, for reasons that are not known.

Regulated mRNA turnover The coexpression of transcript levels is not only controlled by mRNA synthesis but is also mediated by RNA decay, and the details of regulated mRNA degradation are beginning to unfold. Multiple pathways of mRNA turnover exist in yeast, including poly-adenylation-mediated mRNA degradation (apparently the primary route of mRNA turnover in dividing cells), nonsense-mediated decay (which degrades aberrant as well as normal transcripts), and 3' to 5' transcript degradation (reviewed in (McCarthy 1998; Wilusz et al. 2001). In the polyadenylation-mediated degradation pathway, upon removal of the tail by specific yeast factors, the 5' 7-methylguanosine cap of the transcript is removed to allow 5' to 3' exonucleases to degrade the mRNA. Many factors have been implicated in mRNA stabilization and decay; a few examples will be presented here, as they pertain to the regulation of genes that participate in the ESR.

In yeast, and likely in other organisms as well, transcripts that encode functionally-related proteins are coordinately degraded. In a study by Wang *et al.* (2001), the decay rates of the genomic transcripts of dividing cells were characterized after rapid inhibition of nascent protein synthesis [Wang, 2001 #209]. Upon shifting cells to the nonpermissive temperature required to inactivate an RNA Pol II mutant protein (*rpb1-1*), the mRNA decay rates were measured. The study showed that functionally-related transcripts, especially those that encode proteins involved in multi-subunit complexes,

share highly similar decay rates, indicating that transcript degradation is coregulated. The vast majority of these transcripts appeared to be degraded by the poly-adenylation-dependent pathway, as the disappearance of the polyA tail occurred before degradation of the rest of the transcripts [Wang, 2001 #209]. In terms of the genes that participate in the ESR, genes that are induced in this program *versus* those that are repressed show differential decay rates: under the conditions studied, genes that are repressed as part of the ESR were degraded on average 4X faster than genes induced in the ESR. Because the experiment required a 37°C temperature shift to inactivate transcription, it is possible that the differential half lives observed for the genes induced *versus* repressed in the ESR are specific this stressful transition, which would normally trigger gene expression changes indicative of ESR initiation.

In mammalian cells, the half lives of stress-induced transcripts can be altered by RNA binding proteins that affect transcript stability. A number of cytokines, including TNF? , contain AU-rich sequences known as AREs in their 3' untranslated region (UTR) that destabilize the transcripts (Chen and Shyu 1995). In response to specific conditions, the half lives of these transcripts can be extended *via* binding of the ARE sequences by different proteins as well as through an independent mechanism mediated by the stress-kinase p38 (Kontoyiannis et al. 1999; Winzen et al. 1999; Dean et al. 2001). ARE sequences appear to play a role in yeast transcript stability as well. Vasudevan and Peltz (2001) showed that the transcript encoding the translation elongation factor TIF51A, which contains multiple 3' AREs, has different half lives in glucose- *versus* glycerol-containing medium (Vasudevan and Peltz 2001). Introduction of the TNF? 3'UTR to a reporter construct conferred carbon source-regulated mRNA turnover, suggesting that the 3' ARE sequences in the reporter, and perhaps also the TIF51A transcript, contribute to the regulated mRNA turnover. Stabilization of the reporter construct was proposed to be dependent on Pub1p, an RNA binding protein homologous to one of the mammalian ARE-binding factors (Anderson et al. 1993; Matunis et al. 1993): Pub1p bound to the 3' AREs of the reporter construct *in vitro*, and deletion of *PUB1* was correlated with decreased levels of the transcript *in vivo* (Vasudevan and Peltz 2001). Other genes that are repressed in the ESR are probably also controlled by this mechanism, however because the ARE sequence is commonly found in 3' UTRs in the genome (~2 per gene), it is difficult to predict additional ARE-regulated genes based the presence of ARE sequences alone.

Other genes repressed in the ESR show differential mRNA stability in response to stress, particularly during the diauxic shift transition. Among these are three genes that are repressed in the ESR (*RPL10, RPS15, ARO4*). As demonstrated by Albig and Decker, the half lives of these transcripts were reduced as the cell went through the diauxic shift transition and when cells were exposed to rapamycin, an inhibitor of the Tor1p kinase (Albig and Decker 2001). The degradation of a reporter transcript was abrogated in a rapamycin-resistant *tor1* mutant strain, indicating that Tor1 signaling affects mRNA degradation under these conditions. The mechanism of the Tor1-mediated mRNA decay is unknown, however because the *ARO4* transcript showed an increased rate of polyA shortening, it was proposed that the regulation was through poly-adenylation-mediated degradation. In response to rapamycin, the increase in mRNA decay rates was transient: after ~60 min of rapamycin treatment the cells adapted to the drug and the half lives of these transcripts returned to levels seen in untreated cells. This

observation is consistent with the transient changes in transcript levels of genes that participate in the ESR following stressful environmental changes.

Msn2p and Msn4p In terms of the regulators of ESR gene expression, perhaps the best characterized are the transcription factors Msn2p and Msn4p. Originally isolated as high copy suppressors of *SNF1* deletion (Estruch and Carlson 1993), these related factors were subsequently shown to bind the STRE promoter sequence that had been implicated in a general stress response in yeast (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). Indeed, Msn2/4p are involved in the induction of many of the genes that are induced in the ESR in response to stressful conditions, and double deletion of these factors diminishes the induction of ESR genes and renders cells sensitive to a variety of environmental stresses (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996; Boy-Marcotte et al. 1998; Gasch et al. 2000; Amoros and Estruch 2001). Although the factors are often considered to be redundant, Msn2/4p appear to affect gene induction in a manner that is gene specific and condition specific, as discussed below. Because these factors play such a large role in the regulation of the genes induced in the ESR, significant discussion of their properties will be presented here.

Characterization. Msn2p and Msn4p share 32% amino acid identity, particularly in their DNA binding domains which are nearly identical, and they belong to the zinc-finger family of transcription factors (Estruch and Carlson 1993). Both factors bind the STRE sequence (CCCCT) with specificity *in vitro*, and both can mediate gene induction from a plasmid construct containing the STRE promoter element, indicating that this sequence is sufficient for Msn2/4p-dependent gene induction (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). *In vitro* binding of the STRE by either factor does not require stress treatment, as Msn2p and Msn4p bind to the STRE whether the proteins are recovered from cell extracts of stressed or healthy cells (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996). This observation revealed that the proteins are activated in a manner that does not affect their DNA binding properties. It was subsequently realized that the activity of the transcription factors is dependent on their localization: Gorner *et al.* (1998) used epitope-tagged versions of these factors to demonstrate that in the absence of stress, Msn2p and Msn4p reside in the cytosol, however in response to stressful environments the factors are rapidly translocated to the nucleus (Gorner et al. 1998). Within minutes of exposure to temperature shock, hyper-osmolarity, salt stress, ethanol exposure, and glucose starvation, the majority of Msn2p and Msn4p within the cell accumulates in the nucleus. After the cells are returned to their standard growth conditions, Msn2p and Msn4p relocalize to the cytosol within minutes, even in the absence of nascent protein synthesis, indicating that translocation of the proteins is reversible.

Regulation of Msn2/4p nuclear localization. That the nuclear translocation of Msn2p is independent of protein synthesis pointed to a post-translational regulatory mechanism governing the relocalization. Induction of STRE-regulated genes is known to be negatively affected by Protein Kinase A (PKA) signaling (Marchler et al. 1993; Boy-Marcotte et al. 1998), and therefore Gorner *et al.* characterized the involvement of this pathway in mediating Msn2p and Msn4p localization. In cells defective in PKA signaling, Msn2p and Msn4p were shown to be primarily nuclear, whereas cells with abnormally high cAMP showed nuclear exclusion of the factors, even in the presence of

environmental stress. These data revealed that PKA signaling negatively affects Msn2p and Msn4p-dependent signaling by restricting the factors to the cytosol. The amino-termini of both Msn2p and Msn4p contain multiple putative phosphorylation sites, and indeed mutation of the serine residues in these sites on Msn2p results in a constitutively-nuclear protein (Gorner et al. 1998). Thus, it was proposed that PKA might directly phosphorylate Msn2p and Msn4p to trigger cytoplasmic relocalization of the proteins. Contradictory to this original model, it was subsequently observed by Garreau *et al.* (2000) that under standard growth conditions, both Msn2p and Msn4p exist in a phosphorylated state but they become hyper-phosphorylated in response to multiple stresses (Garreau et al. 2000). Addition of cAMP after cellular stress reversed the hyper-phosphorylation state and triggered relocalization of the factors to the cytosol. Thus, it now appears that the inhibitory effect of PKA signaling leads to the dephosphorylation of Msn2p and Msn4p to trigger their nuclear exclusion. The PKA-dependent phosphatase that acts on Msn2p and Msn4p is at present unknown.

The stress-induced relocalization of Msn2p is entirely dependent on a ~300 amino acid region of the protein that contains the putative phosphorylation sites, since fusion of this protein sequence onto an SV40 nuclear localization signal results in PKA- and stress-regulated translocation of the chimera, even in the absence of the remaining Msn2p protein sequence (Gorner et al. 1998). The corresponding domain in Msn4p was not extensively studied, however multiple putative phosphorylation sites are conserved in this region of Msn4p. These details led Gorner *et al.* to propose that this domain may bind to a cytosolic protein that serves to anchor Msn2p and Msn4p in the cytosol under standard growth conditions. It was recently shown that in the absence of cellular stress, Msn2p and Msn4p interact with the consitutively-cytosolic protein Bmh2p (Beck and Hall 1999). Bmh2p is homologous to mammalian 14-3-3 proteins that mediate the localization of signaling proteins, and it and its paralog Bmh1p have been implicated in RAS-dependent signaling and pseudohyphal development in yeast (Gelperin et al. 1995; Roberts et al. 1997; Roth et al. 1999). Under standard growth conditions, Bmh2p can be isolated in a complex with Msn2p or Msn4p, however in response to starvation or inhibition of the Tor1p kinase, the association between the proteins is not observed, and Msn2p accumulates in the nucleus (Beck and Hall 1999). Although it is not known whether the interaction with Bmh2p mediates the cytosolic localization of Msn2p or merely correlates with it, it is interesting to note that mammalian 14-3-3 proteins bind phosphoserine residues in proteins (Muslin et al. 1996; Bertram et al. 1998; Muslin and Xing 2000), hinting that Bmh2p may bind to Msn2p and Msn4p in a manner dependent on the precise phosphorylation state of these proteins.

Differential role and regulation of Msn2p *versus* Msn4p. Msn2p and Msn4p are often treated as 'redundant' transcription factors, however these proteins show subtly different behavior in cells. It has been observed that for many of the known STRE-regulated stress genes, deletion of *MSN2* results in decreased gene induction in response to stress, whereas deletion of *MSN4* often has no result on gene expression (Martinez-Pastor et al. 1996; Schmitt and McEntee 1996; Treger et al. 1998; Amoros and Estruch 2001). However, in the absence of Msn2p, Msn4p clearly contributes to gene induction, as deletion of both *MSN2* and *MSN4* results in significantly weaker gene induction relative to the *MSN2* single deletion mutant (Treger et al. 1998; Garreau et al. 2000). These results indicate that Msn2p and Msn4p may have different functions in the cell.

Consistent with this observation, the proteins are regulated differently. First, in response to stressful environments *MSN4* is induced as part of the ESR (DeRisi et al. 1997; Gasch et al. 2000), and at least in response to glucose starvation, the increased expression parallels increased levels of Msn4p, whereas neither *MSN2* nor its gene product increases in response to stressful environments (Garreau et al. 2000). Second, Garreau *et al.* (2000) showed that the stress-induced alterations in protein phosphorylation were different for Msn2p *versus* Msn4p, indicating that the proteins are differentially phosphorylated. Thus, although Msn2p and Msn4p can both activate gene induction through the STRE promoter element, the proteins appear to be regulated differently and may play unique roles in the regulation of specific genes induced in the ESR.

Targets of Msn2p and Msn4p. The role that Msn2p and/or Msn4p (herein referred to as Msn2/4p for simplicity) play in stress-responsive gene induction varies for each gene target as well as for the specific environmental conditions. For example, genes induced in the ESR show varying dependence on Msn2/4p, indicating that collectively these factors play different regulatory roles on a gene-by-gene basis. Genomic expression in *msn2msn4* mutant cells responding to heat shock or hydrogen peroxide treatment revealed that approximately 60% of the ~300 genes induced in the ESR were affected by the double deletion, and these genes fell into three groups (Gasch et al. 2000). One group of genes, including many of the previously known targets of the factors, were largely dependent on Msn2/4p for induction in response to both heat shock and hydrogen peroxide treatment, while a second group was partially dependent on the factors in response to both stresses. The induction of a third group of genes was dependent on Msn2/4p in response to heat shock, but was unaffected by deletion of the factors in response to hydrogen peroxide, revealing that Msn2/4p are conditionally involved in the regulation of these genes. (It should be noted that for the vast majority of these genes, residual gene induction occurred in the absence of Msn2/4p, indicating the involvement of additional regulators.) Most of the genes that were affected by deletion of *MSN2* and *MSN4* were also responsive to overexpression of the factors, and approximately 80 additional genes that were unaffected in the *msn2msn4* strain responding to heat shock or hydrogen peroxide were induced by *MSN2* or *MSN4* overexpression, revealing even more gene targets. These details show that Msn2/4p contribute to the induction of ESR genes to varying degrees, in a manner that is different for each gene and also dependent on the specific environmental features. The condition-specific involvement of Msn2/4p is supported by the observation that the pattern of hyper-phosphorylation of each protein is unique in response to different environmental stresses, suggesting that the proteins are regulated in different ways in response to different stimuli (Garreau et al. 2000). One possibility is that Msn2/4p are phosphorylated by different, condition-specific protein kinases; whether the different hyper-phosphorylation patterns result in different activation states of Msn2/4p or similarly activate the factors in all cases is not known.

Of the genes affected by Msn2/4p, roughly 95% contain at least one canonical STRE within 800 bp upstream of the open reading frame, and many of the genes contain multiple STREs. In fact, the level of transcript induction is roughly proportional to the STRE copy number in the genes' promoters: genes with multiple STREs show stronger induction in response to a number of environmental transitions, relative to Msn2/4p-regulated genes with one or zero STRE sequences (Figure 7, A. Moses and M.B. Eisen, personal communication). The presence of one STRE promoter element is sufficient for

Msn2/4p-regulation of a plasmid construct, however mere presence of this sequence in genomic promoters does not necessarily result in Msn2/4p-mediated transcriptional induction. Whereas ~3,600 genes in the yeast genome contain at least one STRE within 800 bp upstream of the open reading frame, only ~200 genes are affected by *MSN2/4* deletion or overexpression, and only ~300 genes display stress-responsive gene induction profiles. This is consistent with observations of other known regulatory sequences, for which only a fraction of those present in the genome are bound by their respective factors (Ren et al. 2000; Iyer et al. 2001; Lieb et al. 2001; Simon et al. 2001). This suggests that many of these sequences, including many of the genomic STRE sequences, are not bound by Msn2/4p, perhaps because they are obscured in the genome by other DNA binding proteins or by the local chromatin conformation. Other genes that appear to be affected by Msn2/4p do not contain STRE promoter elements; as no other similarities in these promoters are apparent, the affect of Msn2/4p on these genes, be it direct or indirect, remains unclear.

Condition-specific transcriptional induction Msn2/4p are not always required for induction of the ESR genes, suggesting that other regulatory mechanism come into play under specific environmental conditions. Indeed, it appears that many different condition-specific transcription factors are involved in regulating subsets of genes in the ESR. This was clearly shown in a study by Rep *et al* (1999), which characterized the involvement of four transcription factors, Msn1p, Msn2p, Msn4p, and Hot1p, in inducing three genes that were subsequently identified as ESR genes (*GPD1*, *HSP12*, and *CTT1*) (Figure 8) (Rep et al. 1999). In the case of *GPD1*, which encodes an isozyme of glycerol dehydrogenase, its induction was mainly governed by Hot1p in response to osmotic shock, but controlled by Msn2/4p in response to heat shock in a manner unaffected by *HOT1* deletion. Induction of the cytosolic catalase gene *CTT1* was controlled by Msn2/4p and Msn1p following osmotic shock, evident by the fact that deletion of *MSN2/MSN4* or *MSN1* muted the induction of *CTT1*; however, in response to heat shock *CTT1* induction appeared to be almost entirely controlled by Msn2/4p, with little contribution from Msn1p. In contrast to these genes, the induction of *HSP12* was controlled by Msn2/4p in response to both osmotic and heat shock. These results reveal that *GPD1* and *CTT1* are regulated by different transcription factors under different conditions and each of the genes displays different dependencies on the transcription factors studied.

Similar results have been observed for other genes and transcription factors. A subset of genes induced in the ESR, including those encoding protein folding chaperones, are super-induced in response to heat shock relative to other ESR genes; many of these genes contain promoter elements recognized by the heat shock transcription factor Hsf1p as well as the STRE bound by Msn2/4p, implicating these factors in the genes' regulation. Indeed, Hsf1p and Msn2/4p are involved in regulating many of these genes, although the factors appear to play different roles in response to different stresses. Whereas the induction of *HSP26* and *HSP104* is primarily controlled by Hsf1p in response to heat shock, their induction is largely dependent on Msn2/4p in response to carbon starvation, oxidative stress, or osmotic shock (Treger et al. 1998; Amoros and Estruch 2001). Analogous to the protein folding chaperones, a subset of ESR genes that have been implicated in the response to oxidative stress are super-induced in response to

conditions that inflict oxidative damage; these genes are induced by the oxidation-activated Yap1p transcription factor in response to hydrogen peroxide, but they are regulated by Msn2/4p following heat shock, independent of Yap1p (Gasch et al. 2000). Together, these data show that subsets of genes in the ESR are regulated by different transcription factors under different conditions, namely Hot1p during osmotic shock, Hsf1p following heat shock, and Yap1p in response to oxidative stress. In contrast, all of the genes tested were affected in some way by Msn2/4p, although the precise role of the factors varied for each gene in response to the different conditions tested.

In all of the studies mentioned above, residual gene induction was observed in strains lacking individual transcription factors, indicating that other regulators can supplement for the absent factors (Treger et al. 1998; Rep et al. 1999; Gasch et al. 2000; Amoros and Estruch 2001). For example, Rep *et al.* showed that in an otherwise wild-type background, deletion of the transcription factor Hot1p had no affect on the induction of *HSP12* in response to either heat shock or osmotic shock; however, in the absence of the primary *HSP12* regulators Msn2/4p, Hot1p significantly contributed to the residual induction of the gene following osmotic shock. A quadruple mutant lacking *HOT1*, *MSN1*, *MSN2*, and *MSN4* still showed residual induction of *HSP12* in response to osmotic stress, suggesting still other regulators. *HSP12* is known to be induced by Hsf1p in the absence of Msn2/4p (Treger et al. 1998), and perhaps this factor contributes to the residual induction of *HSP12* in the quadruple transcription factor mutant. Conversely, the involvement of Msn2/4p in regulating the ESR genes *HSP26*, *HSP78*, *HSP104* and *UBI4* following heat shock could only be detected in the absence of Hsf1p (Treger et al. 1998; Simon et al. 1999). Thus, although certain transcription factors are not required for the induction of specific genes in wild-type cells, those factors play a significant role in transcriptional regulation in the absence of the genes' primary regulators.

Condition-specific cellular signaling Consistent with the condition-specific transcriptional regulation of genes induced in the ESR, the coordinate expression of genes induced and repressed in this program is controlled by different upstream signaling pathways depending on the environmental conditions. For example, the genomic expression pattern in wild-type and mutant cells responding to DNA-damaging agents reveals that the Mec1 pathway is involved in regulating the ESR (Gasch et al. 2001). Mec1p is a protein kinase, related to phospho-inositol kinases, that is activated in response to DNA damage to phosphorylate downstream kinases that govern cell-cycle arrest and gene expression changes (refs). In response to DNA damaging agents, wild-type cells respond by inducing a subset of genes specifically enlisted to repair the resulting cellular damage and by initiating the ESR (Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Lee et al. 2001b)others??. However, cells defective in Mec1 signaling fail to induce the DNA damage-specific genes and also fail to properly initiate the ESR: the mutant cells lacking Mec1p or its downstream kinase Dun1p displayed muted expression changes of the genes that participate in the ESR, with an almost complete lack of ESR gene repression (Gasch et al. 2001). In contrast, in response to heat shock deletion of *MEC1* or *DUN1* had no significant effect on the initiation of the ESR. These results reveal that initiation of the entire ESR is controlled by the Mec1 pathway in response to DNA-damaging agents but by another mechanism following heat shock.

Other signaling pathways have also been implicated in the condition-specific regulation of genes in the ESR. The Protein Kinase C (PKC) MAPK pathway in yeast is activated by a number of conditions, including heat shock, secretion defects, and cell wall damage (ref, Warner, Jung & Levin, others). In response to secretion defects, the PKC pathway governs the reduction in rRNA, tRNA, and RP transcripts; however, PKC signaling is not required for this repression in response to other conditions such as amino acid starvation (Mizuta et al. 1998; Li et al. 1999; Nierras and Warner 1999; Li et al. 2000; Miyoshi et al. 2001). Given the stereotypical expression patterns of genes in the ESR, it is likely that the PKC pathway governs initiation of the entire program in response to certain conditions. Indeed, cells lacking Slt2p, the terminal kinase on the PKC pathway, show muted induction of ~70% of the genes induced in the ESR, although genes repressed in the ESR are unaffected by *SLT2* deletion (A.P. Gasch and P.O. Brown, unpublished data). That expression of the repressed ESR genes was unaffected by Slt2p is consistent with evidence by Li *et al.* (2000), which showed that RP gene repression is controlled by the top kinase in the pathway, Pkc1p, but does not involve the known downstream kinases (including Slt2p), leading the group to propose a bifurcation of the PKC pathway. These preliminary details suggest that genes induced in the ESR are regulated by a different branch of the PKC pathway than genes repressed in the ESR.

The high osmolarity-responsive MAPK pathway involving Hog1p has also been implicated in regulating the ESR, and similar to the case of PKC, different branches of the pathway may control the induced *versus* repressed ESR genes. Hog1p is the terminal MAPK in a series of pathways that respond independently to high osmolarity, and activation of the kinase leads to translation arrest and gene expression changes critical to the survival of hyper-osmotic shock (Teige EF2 ref) (see Chapter X). In response to salt- or sorbitol-mediated hyper-osmotic shock, many of the genes induced in the ESR show muted and prolonged induction in cells lacking *HOG1* (Rep et al. 2000), S.M. O'rourke and I. Herskowitz, personal communication); in contrast, the genes that are repressed in the ESR show stronger, prolonged patterns of repression in osmotic-shocked cells compared to the wild type (S.M. O'Rourke and I. Herskowitz, personal communication). Unlike the *hog1* mutant, O'Rourke and Herskowitz demonstrated that cells lacking *STE11* and *SHO1*, encoding two upstream kinases thought to act in parallel to activate Hog1p, showed reduced expression changes for both the induced and repressed ESR genes (S. O'rourke and I. Herskowitz, personal communication). Thus, genes induced in the ESR *versus* those that are repressed in the program appear to be controlled by different branches of the Ste11/Sho1p-dependent signaling pathways in response to osmotic shock. The prolonged ESR gene expression changes seen in the *hog1* mutant suggest that these partially-unresponsive cells may be experiencing a higher level of internal stress, consistent with the known sensitivity of the mutant to hyper-osmotic shock (Brewster et al. 1993).

Other regulatory systems have been tentatively linked to ESR regulation. The Snf1p kinase is activated by carbon starvation and leads to the derepression of many genes involved in respiration (for review see (Carlson 1999). Snf1p also regulates a number of processes associated with the ESR, including glycogen accumulation and autophagy (Cannon et al. 1994; Wang et al. 2001a), hinting that the kinase may play some role in ESR initiation. Furthermore, deletion of the kinase is suppressed by high-copy expression of *MSN2* or *MSN4*, although the mechanism of the suppression is not

entirely clear. Recently, a role for the Pho85 kinase, a proposed antagonist of Snf1p-dependent effects (Huang et al. 1998; Wang et al. 2001a; Wang et al. 2001b)(ref), has been implicated in ESR regulation. Chemical inactivation of the kinase leads to initiation of the ESR relative to control experiments, however whether the stress response is directly controlled or indirectly affected by rapid inactivation of Pho85p is not known (Carroll et al. 2001). Despite these details, the precise role of Snf1p and Pho85p in ESR regulation remains to be decisively demonstrated.

The MEC-, PKC-, HOG-, and likely other pathways are required for proper initiation of the ESR in response to specific environmental stresses, but other signaling systems have been implicated in the suppression of the ESR. The best characterized is the PKA system (see Figure 5). When starved cells are supplemented with glucose, a spike in intracellular cAMP levels (Mazon et al. 1982; Francois et al. 1988) triggers PKA activity (Jiang et al. 1998), and correlated with the resumption of cell growth is the increased expression of genes involved in protein synthesis and the decreased expression of Msn2/4p target genes (Marchler et al. 1993; Klein and Struhl 1994; Gorner et al. 1998; Thevelein and de Winde 1999; Norbeck and Blomberg 2000). These observations suggest that PKA activity suppresses the ESR in response to nutrient repletion to promote the resumption of normal cell growth. The negative effect of PKA signaling on ESR initiation is further evident by the fact that artificially high intracellular cAMP levels can suppress the ESR, even in the presence of environmental stress (Klein and Struhl 1994; Neuman-Silberberg et al. 1995; Gorner et al. 1998; Garreau et al. 2000). Whether PKA signaling provides a generalized mechanism of ESR suppression following stress relief, or is specific to nutrient repletion, is not known. An increase in intracellular cAMP is apparently not a general feature of stress relief, indicating that at least the cAMP spike seen following nutrient supplementation is specific to this condition (M. Jacquet, personal communication). However, that a number of genes affecting PKA signaling are induced in the ESR in response to environmental stress argues for a more general role of the pathway. Because inappropriate activation of the ESR is likely detrimental to cell growth, one aspect of the ESR is to prepare for the suppression of the program once the cell has adapted to its new conditions. The induction of the PKA signaling components as part of the ESR may represent this preparation for ESR suppression. Alternatively, the induction of PKA genes may serve some other purpose: confusingly, cells lacking the catalytic subunits of PKA are unable to repress RP gene expression, a feature seen when the ESR is initiated, in response to glucose starvation (Ashe et al. 2000), arguing for a role for the PKA pathway in proper activation of the ESR. These complexities hint that PKA signaling components may have multiple roles in mediating ESR gene expression.

Similar to PKA signaling, activity of the TOR pathway has also been implicated in ESR suppression. The Tor1 kinase negatively regulates Msn2/4p by triggering cytosolic relocalization of the factors, while inhibition of Tor1p signaling prevents translational arrest and repression of genes in the ESR in response to rapamycin and glucose starvation (Barbet et al. 1996; Beck and Hall 1999; Powers and Walter 1999). Again, exposure of cells to stressful environments leads to the induction of the gene encoding Tor1p as part of the ESR, in a manner dependent on Msn2/4p, raising the possibility that Tor-dependent signaling plays a more general role in mediating the ESR, perhaps as a mechanism of ESR suppression following adaptation to new environments.

Advantages to the complex regulation of ESR gene expression The details presented above reveal the complexities of the regulation of ESR gene expression: changes in transcript abundance are mediated at the level of transcription initiation, chromatin structure, and mRNA stability, and these processes are controlled by different regulators in response to different conditions. Such a complicated system of regulation provides a number of advantages to the cell. First, this system allows specificity and precision in the cellular response to each new environment. By controlling ESR gene expression with many different factors, the cell can customize the overall initiation of the program to the exact features of the new environment. For example, different subsets of ESR genes are particularly critical to survival in response to specific conditions, and therefore under the relevant circumstances those genes are controlled by specialized factors that guarantee their appropriate expression. Thus, the cell can sensitively control the precise levels of ~900 transcripts in the ESR by altering the context of a limited number of signaling factors which converge on the ESR genes to result in a unique overall pattern of ESR initiation. This system represents a relatively simple mechanism of providing a wide range of subtly different ESR expression programs by modulating the relative activities of a small number of upstream regulators.

A second advantage of this complicated regulatory system is that it provides a back-up mechanism of ESR initiation. In the absence of a primary regulator of a given ESR gene, other signaling factors can supplement for the regulator's activity to provide residual expression changes. Some of the genes discussed here are regulated by at least five transcription factors, and other mechanisms of transcript regulation, such as regulated mRNA turnover, probably also contribute to the stress-induced expression changes. The cell has evolved this system to effectively guarantee initiation of the ESR in response to stressful environments, underscoring the importance of this program in surviving environmental fluctuations.

A model for the orchestration of cellular responses to environmental stresses

The details presented in this review can be compiled to generate a model for the mechanism that cells use to orchestrate cellular responses to stressful environmental transitions. When a cell is transferred to a new environment for which its internal system is not optimized, specific cellular challenges arise. These features are detected by specific sensory systems (not discussed in detail here) which activate specialized signaling pathways to transmit a cellular signal. The upstream signaling factors activate multiple downstream protein kinases, each of which in turn likely activates a host of proteins that mediate cellular responses such as cell-cycle arrest, translation inhibition, gene and protein expression changes, and enzymatic alterations. In terms of the gene expression changes, each specialized signaling pathway probably activates a variety of factors that affect gene expression. Some of these regulators trigger expression changes in genes that are specifically enlisted to combat the unique cellular challenges initially detected by the cell. At the same time, these and other factors collectively alter the expression of ~900 genes that participate in the ESR, initiated to protect critical features of the internal homeostasis in the cell in times of stress. Thus, each cellular response is precisely orchestrated to result in a unique cellular program that will ensure survival of the cell in the new environment.

Immediately after cells are shifted to a stressful environment, they respond by arresting growth and protein synthesis while triggering large changes in gene expression; however, over time the cell often adapts to the new conditions, and the alterations in genomic transcript levels subside as growth and translation resume. One model for the role of the transient gene expression changes is that large initial alterations in gene expression may promote the rapid adjustment in the corresponding gene products, and their activities, to new steady-state levels. A model for order of events that occur during the adaptation phase is shown in Figure 9, using the response to hyper-osmotic shock as an example. When cells are initially shifted to medium of high osmolarity, substantial cellular defects arise because the cells are not prepared to deal with this environment. These defects trigger a strong cellular signal that leads to the phosphorylation and nuclear accumulation of Hog1p, followed by large changes in gene expression that are mediated by Hog1p and other factors (see Chapter X details; (Parrou et al. 1997; Reiser et al. 1999; Rep et al. 1999). As transcripts critical to cell survival become translated the activities of the corresponding gene products begin to counteract the cellular defect, reducing the amount of stress in the cell and diminishing cellular signaling related to the stress. Subsequently, Hog1p relocalizes the cytoplasm and gene expression levels adjust to their new steady-state levels. Thus, although Hog1p activation and gene expression changes are transient, the levels and activities of the corresponding proteins steadily increase until they reach the new steady-state levels required for growth at the new conditions.

Two models can account for the mechanism of transient gene expression changes. One model is that after strong initiation of the ESR, one or more regulatory systems are activated to suppress the response until it reaches the appropriate state. This situation would require the initial activation of positive regulators of ESR initiation, followed by the activation of negative regulators of the program. Alternatively, adaptation of ESR gene expression may arise due to attenuation of cellular signaling that initiates the response, coupled with a constitutively-active mechanism of ESR suppression. In this model, cellular signaling immediately following a stressful environmental shift would override the mechanism that normally suppresses the ESR, leading to a strong initiation of the stress response. As the cell adapts to its new environment, the corresponding stress signal would abate, and the precise levels of ESR gene expression would be dictated by the balance of signaling through the positive (initiating) and negative (suppressing) signals. This is model is consistent with the fact that cells lacking PKA activity have constitutively nuclear Msn2/4p, even under optimal growth conditions, suggesting that PKA is involved in mediating ESR suppression in the absence and presence of environmental stress.

Concluding remarks

Many details remain to be elucidated regarding the role and regulation of the ESR during the cellular adaptation to environmental changes. How initiation of the ESR contributes to cellular resistance to various stresses is a critical question in understanding the role that this program plays in the yeast life cycle. The importance of the program is suggested by the complicated mechanism of regulation of ESR gene expression, indicating that the cell goes to great lengths to ensure that the expression of the program is delicately balanced to the demands of the cell. A variety of regulatory factors have been implicated in regulating subsets of ESR genes under specific conditions, and more

will certainly emerge. Elucidating the interplay between these factors will contribute to our understanding of how the cell regulates ESR gene expression. Furthermore, detailed characterization of the signaling networks that govern these factors will provide insights into the mechanisms that the cell uses to precisely orchestrate the many features of its response, including changes in genomic expression programs, inhibition of protein synthesis, arrest of the cell cycle, and the subsequent resumption of these processes as the cell adapts to its new environment.

Another important consideration in the study of organismal responses to diverse environmental changes is the degree to which such a common stress response is conserved in other organisms. Preliminary results indicate that wild isolates of *S. cerevisiae* initiate the ESR in response to environmental changes, similar to the well-studied lab strains (S. Harel, B. Carlson, J.C. Fay, A.P.Gasch, M.B. Eisen, unpublished results). It will be interesting to learn whether the distantly related fission yeast *S. pombe* utilizes a similar response; current understanding of the *S. pombe* response to different stresses suggests that this yeast, in contrast to *S. cerevisiae*, utilizes a single MAPK protein kinase that is activated by a wide variety of stresses, and thus it will be especially interesting to compare the differences in the stress-dependent regulation of genomic expression in these organisms [Millar, 1995 #210][Shiozaki, 1995 #211][Degols, 1996 #212][Shieh, 1997 #213]. Answering these questions will help us understand the remarkable ability of yeast and other organisms to survive stressful variations in their environments.

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FIGURE LEGENDS

Figure 1. Gene expression patterns in the ESR. The average gene expression changes of the genes induced (—■—) and repressed (—●—) in the ESR in response to 25°C to 37°C heat shock, 0.3 mM Hydrogen peroxide, 1.5 mM diamide, 1M sorbitol, 0.02% Methylmethane sulfonate, and 170 Gray of ionizing radiation. The same scale is used for all of the plots shown. Data taken from Gasch 2000 and Gasch 2001. The complete list of the genes that participate in this response can be found at http://www-stanford.edu/yeast_stress.

Figure 2. Initiation of the ESR is often transient. Average gene expression changes of the genes induced (—■—) and repressed (—●—) in the ESR in response to a 25°C to 37°C heat shock and a 29°C to 33°C heat shock. Data taken from Gasch 2000.

Figure 3. Reciprocal expression of the ESR genes in response to reciprocal environmental changes. The average gene expression changes of genes induced (—■—) and repressed (—●—) are shown as cells responded to a 25°C to 37°C shock (A, left panel), 37°C to 25°C shock (A, right panel), a shift from YPD medium to YPD supplemented with 1M sorbitol (B, left panel), and YPD with 1M sorbitol to standard YPD medium (B, right panel).

Figure 4. ESR genes involved in carbohydrate metabolism. Genes induced as part of the ESR that are involved in glucose import, trehlose and glycogen metabolism, the pentose phosphate shuttle, glycolysis, fatty acid metabolism, and respiration are shown in bold.

Figure 5. Schematic diagram of PKA signaling in regard to ESR suppression. In response to increased cAMP levels, the inhibitor of PKA signaling (Bcy1p) binds cAMP to release the active complex, composed of two of three PKA subunits (Tpk1p, Tpk2p, Tpk3p) (refs). PKA activity suppresses the ESR by affecting regulators that lead to the induction of protein synthesis genes and by triggering nuclear export of Msn2/4p and decreased expression of their targets. In response to stressful environmental stimuli, the ESR is initiated, and nuclear-localized Msn2/4p induces genes in the ESR, including the factors shown here that inhibit PKA signaling.

Figure 6. Subsets of ESR genes are super-induced under certain conditions. (A) Each bar indicates the average expression and standard deviation of 268 genes induced in the ESR as measured in one timepoint during the response to 25°C to 37°C heat shock (5, 15, 30, 60 min), 0.3 mM Hydrogen peroxide (10, 20, 30, 40, 50, 60, 80, 100, 120, 160 min), 2.5 mM DTT (15, 30, 60, 120, 480 min), 1M sorbitol (5, 15, 30, 45, 60 min), amino acid starvation (0.5, 1, 2, 4, 6 h), and progression into stationary phase (2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120). (B) The average expression and standard deviation of 13 genes that are super-induced in response to hydrogen peroxide and (C) of 17 genes that are super-induced in response to heat shock are shown, as described for (A).

Figure 7. Msn2/4p-dependent induction is proportional to the STRE copy number. Approximately 180 genes whose induction in response to heat shock was dependent on Msn2/4p were binned into ten groups based on the number of STRE (CCCCT) elements within 800 basepairs upstream of each open reading frame. The average induction and standard deviation of genes with 0 to 10 STRE promoter elements is shown at 5, 15, 30, and 60 minutes after a 25^{o}C to 37^{o}C heat shock. The key in the upper right corner indicates the number of genes present in each bin. Figure kindly provide by Alan Moses, based on the data from Gasch et al. 2000.

Figure 8 Diagram of condition-specific regulators??

Figure 9. Model for the sequence of events during stress adaptation. (A) A schematic diagram for the events that occur during stress adaptation, using Hog1p activation as a model. After initial detection of the stress signal, Hog1p becomes phosphorylated and accumulates in the nucleus (Reiser et al. 1999; Rep et al. 1999) to trigger a transient increase in transcript levels (squiggles), which leads to a steady increase in protein levels (circles). See text for details. (B) Temporal profiles of the changes in kinase activation, transcript levels, protein levels, and metabolite levels, using Hog1p and Gpd1p as an example. In response to osmotic shock, transient changes in Hog1p activation and GPD1 transcript levels lead to a steady increase in the amount of Gdp1 protein and its product, glycerol (Reiser et al. 1999; Rep et al. 1999), Stefan Hohmann, personal communication).

Table 1 Environmental transitions that lead to ESR initiation[a]

Environment	References
Temperature Shocks	
Heat shock	[Boy-Marcotte, 1999 #205](Gasch et al. 2000; Causton et al. 2001)
Cold shock	A.P.Gasch and P.O. Brown, unpublished data
Ethanol Shock	[Alexandre, 2001 #206]
pH Extremes	
Acid	(Causton et al. 2001)
Alkali	(Causton et al. 2001)
Oxidative and Reductive Stress	
Hydrogen Peroxide	[Godon, 1998 #55](Gasch et al. 2000; Causton et al. 2001)
Menadione	(Gasch et al. 2000)
Diamide	(Gasch et al. 2000)
Cadmium	(Momose and Iwahashi 2001)
DTT	(Gasch et al. 2000; Travers et al. 2000)
Hyper-Osmotic Shock	
Sorbitol	(Gasch et al. 2000; Rep et al. 2000; Causton et al. 2001)
Potassium Chloride	(S.M. O'Rourke and I. Herskowitz, personal communication)
Sodium Chloride	(Posas et al. 2000; Rep et al. 2000; Causton et al. 2001; Yale and Bohnert 2001)(S.M. O'Rourke and I. Herskowitz, personal communication)
Starvation	
Progression into Stationary Phase	[Fuge, 1994 #5](Gasch et al. 2000)
Amino Acid Starvation	(Gasch et al. 2000; Natarajan et al. 2001)
Nitrogen Starvation	(Gasch et al. 2000)
Phosphate Starvation	(Ogawa et al. 2000)
Zinc Starvation	(Lyons et al. 2000)
Respiration	
Petite mutants	(Traven et al. 2001)
Diauxic Shift Transition	[Fuge, 1994 #5](DeRisi et al. 1997)
Nonfermentable Carbon Sources	(Kuhn et al. 2001)
Diverse Drug Treatments	(Hughes et al. 2000b)
Long-term Exposure to alpha Factor	(Spellman et al. 1998)

DNA Damaging Agents	
Alkylating Agents	(Jelinsky and Samson 1999; Jelinsky et al. 2000; Gasch et al. 2001; Natarajan et al. 2001)
Ionizing Radiation	(Gasch et al. 2001)
Double-strand Breaks	(Lee et al. 2001b)

[a] This table lists global studies of transcript abundance and protein synthesis levels that implicate initiation of the ESR.

Table 2 Functional categories of genes repressed in the ESR

Category[a]	Number of Genes in the ESR[b]	% of All Yeast Genes in Category[b]
Ribosomal Proteins	132	98%
Ribosome Biogenesis	6	75%
rRNA Processing	26	72%
Nuclear Targeting (Excluding Nuclear Pore Components)	6	60%
Transcription		
RNA Pol I Subunits	7	88%
RNA Pol II Subunits	2	7%
RNA Pol III subunits	8	89%
Shared Polymeras Subunits	5	83%
Cytosolic Translation Factors		
Initiation	20	83%
Elongation	9	100%
Termination	3	100%
tRNA Processing	4	12%
tRNA Synthetases (Cytosolic)	15	68%
Nucleotide Metabolism	11	23%
DNA Replication	6	10%
mRNA Splicing	5	8%
mRNA Decay	3	30%

[a] Functional categories as listed in the *Saccharomyces* Genome Database (Ball et al. 2000).
[b] The categories listed contain ~78% of all of the characterized genes that are repressed in the ESR.
[c] Percent of the total number of genes in each category represented by genes in the ESR.

Table 3. Differentially Regulated Paralogs in the ESR[a].

ESR Gene	non-ESR Paralogs	Function
Metabolism		
HXK1	*HXK2*	Hexokinase
GLK1	*YDR516C*	Glucokinase
PGM2	*PGM1*	Phosphoglucomutase
PFK26	*PFK27*	2-phosphofructokinase
FBP26	*FBP1*	Fructose-2,6-bisphosphatase
GPM2	*GPM1 GPM3*	Phosphoglucerate mutase
GSY2	*GSY1*	Glycogen synthase
GLG1	*GLG2*	Glycogen initiator
NTH1	*NTH2*	Neutral trehalase
GND2	*GND1*	6-phosphogluconate dehydrogenase
GPD1	*GPD2*	Glycerol dehydrogenase
CYC7	*CYC1*	Cytochrome C
Oxidative Stress Defense		
TRX2	*TRX1 TRX3*	Thioredoxin
CTT1	*CTA1*	Catalase
SOD1	*SOD2*	Superoxide Dismutase
HYR1 GPX1	*GPX2*	Glutathione peroxidase
GTT1	*GTT2*	Glutathione transferase
PRX1 YDR453C	*TSA1*	Thiol-specific antioxidant
Protein Folding and Degradation		
SSA3/SSA4[b]	*SSA1/SSA2*[b]	Hsp70 chaperones
SSE2	*SSE1*	Hsp70 chaperones
HUL4	*UBA1,2 RSP5 UBR1*	E1 Ubiquitin ligase
UBC5 UBC8	*UBC1,4,6,9,12,13*	E2 Ubiquitin conjugase
Signaling		
TOR1	*TOR2*	Tor kinase subunits
TPK1, TPK2	*TPK3*	Protein Kinase A catalytic subunits
PDE1	*PDE2*	Phosphodiesterase

[a] Data taken from (Causton et al. 2001; Gasch et al. 2001)

[b] These genes are highly similar and prone to crosshybridization on the microarrays, and therefore their individual expression patterns cannot be distinguished.

Table 4. Factors implicated in regulating genes in the ESR

Factor[a]	Number of ESR genes affected[b]	Conditions[c]
Multifunctional proteins		
Rap1p	137 (23%) repressed ESR genes 13 (5%) induced ESR genes	
Silencing		
Tup1p/Ssn6p	XX repressed ESR genes XX induced ESR genes	
Chromatin remodeling		
Rpd3p/Isw2p	65 (23%) induced ESR genes	
rpd3 mutant	164 (28%) repressed ESR genes	
Transcription factors		
Msn2/4p	251 (88%) induced ESR genes	Diverse conditions
Yap1p	17 (6%) induced ESR genes	Oxidative stress
Hsf1p	unknown	Heat shock
Hot1p	unknown	Osmotic shock
Msn1p	unknown	
Sko1p repressor	unknown	Osmotic shock
Protein Kinase Pathways		
MEC pathway	entire ESR	DNA-damaging agents
HOG pathway	entire ESR	Osmotic shock
PCK pathway	entire ESR likely	Heat shock, Secretion Defects
PKA pathway	entire ESR likely	Nutrient repletion, Others?
TOR pathway	entire ESR likely	Nutrient repletion, Others?
SNF1 pathway	unknown	Glucose starvation
PHO85 pathway	unknown	Glucose repletion

[a] See text for references.
[b] Number and percent of the genes induced (out of 286 total) or repressed (out of 588 total) in the ESR.
[c] Conditions under which each factor is known to be active.